AD________________

AWARD NUMBER: W81XWH-05-1-0445

TITLE: Downregulation of ErbB2 by Perturbing its Endocytic Recycling

PRINCIPAL INVESTIGATOR: Victor W Hsu, M.D.

CONTRACTING ORGANIZATION: Brigham and Women's Hospital
Boston, Massachusetts 02115-6110

REPORT DATE: July 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-07-2006	Annual	22 Jun 2005 – 21 Jun 2006

4. TITLE AND SUBTITLE

Downregulation of ErbB2 by Perturbing its Endocytic Recycling

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0445

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Victor W Hsu, M.D.

E-Mail: vhsu@rics.bwh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Brigham and Women's Hospital
Boston, Massachusetts 02115-6110

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
About 25% of breast cancers arise from the overexpression of ErbB2 that increases its cell surface level on mammary cells to result in enhanced mitogenic signaling. This high surface level of ErbB2 is maintained by efficient endocytic recycling rather than slowed internalization. Although transport efficiency involves the recognition of specific sequences in the cytoplasmic domain of cargo proteins (known as sorting signals) by the cellular sorting machinery, this mechanism is thought not to be applicable for the endocytic recycling pathways. However, this long-held paradigm has been reversed by the recent discovery of sorting signals that function in the endocytic recycling of transferrin receptor. As transferrin receptor has been the model system for studying recycling, a key prediction is that other recycling proteins will also possess sorting signals for their efficient recycling. Thus, we propose to identify the predicted recycling sorting signal(s) in ErbB2 by first mutagenizing its cytoplasmic domain, and then stably expressing these mutants as epitope-tagged forms in the ErbB2-driven SKBR3 breast cancer cell line to assess effect on recycling. We anticipate that residues critical for the efficient recycling of ErbB2 will be identified, with the implication that mechanisms of ErbB2 recycling will be an attractive target in the future therapeutic effort to downregulate ErbB2-driven mitogenic signaling.

15. SUBJECT TERMS
ErbB2, endocytic recycling, sorting signal

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	5	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION

ErbB2 belongs to a family of surface transmembrane receptor tyrosine kinases whose activation drives intracellular mitogenic signaling. Its overexpression leads to tumor transformation that accounts for 25% of human breast cancers. A prevailing paradigm has been that surface ErbB2 is a static pool. Thus, therapies against ErbB2-driven tumors, such as monoclonal antibodies against ErbB2 and the antibiotic geldanamycin, are thought to be effective, because they enhance ErbB2 internalization to decrease its surface level. Significantly, a recent study has reversed this prevailing paradigm by showing that the high surface distribution of ErbB2 is maintained mainly by its efficient endocytic recycling rather than its slowed internalization [1], leading to the prospect that the mitogenic signaling by surface ErbB2 may be decreased by inhibiting its endocytic recycling. In this respect, mechanisms of intracellular transport are highly conserved, with transport efficiency dictated by protein sorting that involves the recognition of specific sequences (known as sorting signals) in the cytoplasmic domain of cargo proteins by the cellular sorting machinery [2]. Thus, we had proposed to identify the predicted recycling sorting signal(s) in ErbB2, as an initial step in determining whether the future targeting of the cellular sorting machinery responsible for endocytic recycling may be a therapeutic approach for those breast cancers that are due to overactive ErbB2 signaling on the cell surface. Specifically,

Task 1. Identify a critical region in the ErbB2 cytoplasmic domain that mediates its efficient endocytic recycling:

a. Generate mutant ErbB2 with progressive truncation of its cytoplasmic domain from the carboxyl terminus and attach epitope tag to this end.
b. Generated SkBr3 breast cancer cell lines that stably express the different mutant ErbB2.
c. Perform the biochemical recycling assay.

Task 2. Identify critical residue(s) in the ErbB2 cytoplasmic domain that mediates its efficient endocytic recycling:

a. Perform alanine-scanning mutagenesis on the mutant ErbB2 found to have reduced endocytic recycling in Task 1.
b. Generated SkBr3 breast cancer cell lines that stably express the different mutant ErbB2.
c. Perform the biochemical recycling assay.

BODY

Work on this project was delayed by the unexpected departure of a post-doctoral fellow who was originally assigned to perform the proposed experiments. A qualified replacement was subsequently identified, but his start was delayed until he had finished his doctorate degree. Ultimately, this set of circumstances resulted in about an 8-month delay in the start of the project after its funding officially started. Recently, we have been given a no-cost extension of an additional 8 months, so that a sufficient period of time can be given to pursue the project properly. As update, we have begun to generate truncation forms of ErbB2 followed by their epitope-tagging, as described for Task 1a, and have initiated the generation of stable cell lines that express these truncation mutants, as described for Task 1b.

KEY RESEARCH ACCOMPLISHMENTS

We have only begun on this project, as described above.

REPORTABLE OUTCOMES

None, thus far.

CONCLUSIONS

We anticipate that we will be able to complete the proposed tasks with the extension given, because we have substantial experience in using the proposed mutagenesis techniques to identify sorting signals on cargo proteins, as exemplified by our identification of these signals in the recycling transferrin receptor (TfR) [3]. In fact, the currently proposed approach to test whether ErbB2 has a recycling sorting signal is based on our previous study on TfR recycling.

REFERENCES

1. Austin, C. D. et al. Endocytosis and sorting of ErbB2 and the site of action of cancer therapeutics trastuzumab and geldanamycin. *Mol Biol Cell* **15**, 5268-82 (2004).
2. Bonifacino, J. S. & Glick, B. S. The mechanisms of vesicle budding and fusion. *Cell* **116**, 153-66 (2004).
3. Dai, J. et al. ACAP1 Promotes Endocytic Recycling by Recognizing Recycling Sorting Signals. *Dev Cell* **7**, 771-6 (2004).

APPENDICES

None